Metabolism of nicotine to cotinine studied by a dual stable isotope method

Objectives: (1) To determine the disposition kinetics of nicotine and cotinine, including the fractional conversion of nicotine to cotinine, (2) to compare the disposition kinetics of deuterium-labeled and unlabeled cotinine, and (3) to develop a pharmacokinetically based method for estimating daily intake of nicotine from cigarette smoking.

Study design: Twenty cigarette smokers received a combined infusion of deuterium-labeled nicotine (d_2) and cotinine (d_4). Six nonsmokers received a combined infusion of unlabeled cotinine, cotinine-d_2 and cotinine-d_4. Daily intake of nicotine was estimated with use of the plasma cotinine concentration during ad libitum smoking, clearance of labeled cotinine, and fractional conversion of nicotine to cotinine.

Results: The kinetics of labeled versus unlabeled cotinine and of cotinine in smokers versus nonsmokers were similar. On average, 72% of nicotine was converted to cotinine, with a range from 55% to 92%. Subjects with lower clearances of nicotine had lower fractional conversion of nicotine to cotinine, indicating that this is the most rapid of the proximate metabolic pathways for nicotine. The equation for estimating daily intake of nicotine from smoking was: D_{nic} (mg/24 hr) = K × (Plasma Cot) (ng/ml), where K averaged 0.08, with a range from 0.047 to 0.102. Individual variability in the clearance of cotinine (coefficient of variation, 27.5%) accounts for more of the variability in K than does variability in the fractional conversion of nicotine to cotinine (coefficient of variation, 12.3%).

Conclusions: Our study provides quantitative data on individual variability in the extent of C-oxidation of nicotine to cotinine and a quantitative perspective on the use of plasma cotinine as an indicator of daily intake of nicotine from tobacco. (CLIN PHARMACOL THER 1994;56:483-93.)

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Nicotine is metabolized primarily by C-oxidation to cotinine.[1] Measurement of cotinine concentrations in blood, urine, or saliva has been widely used to assess whether or not a person uses tobacco, the level of exposure to nicotine in tobacco users, and the level of exposure of nonsmokers to environmental tobacco smoke.[2,3] Levels of cotinine in the body are determined by the rate and extent of generation of cotinine from nicotine and the rate of elimination of cotinine. Thus individual differences in the extent of nicotine metabolism to cotinine and in the clearance of cotinine limit the accuracy of cotinine levels as an indicator of nicotine exposure.

Stable isotope methods are particularly useful in studying nicotine metabolism in cigarette smokers because smokers have natural nicotine and metabolites derived from tobacco in their bodies, significant levels of which may persist for several days after cessation of tobacco use.[4] In addition, low-level environmental contamination with nicotine reduces the accuracy of nicotine measurements at low concentrations. The use of stable isotope–labeled nicotine allows metabolic studies to be performed in the presence of natural nicotine. The stable isotope method is also useful in quantitating biotransformation pathways. The drug and labeled metabolite can be administered simultaneously without concern for the presence of natural drug and metabolite or problems with day-to-day variability when drug and metabolite are administered on separate days.

We report here the use of a combined infusion of

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3′,3′-dideuteronicotine (nicotine-d_2) and 2,4,5,6-tetradeuterocotinine (cotinine-d_4) to determine the disposition kinetics of nicotine and cotinine, including the fractional conversion of nicotine to cotinine, in 20 cigarette smokers. The resultant data were used, along with plasma cotinine concentrations measured during ad libitum cigarette smoking, to estimate daily intake of nicotine from cigarette smoke. To validate the use of stable isotopes for cotinine metabolic studies, we compared the disposition kinetics of labeled and unlabeled cotinine in a group of nonsmokers. The disposition kinetics of nicotine-d_2 have been shown previously to be similar to the kinetics of natural nicotine.[5]

METHODS

Subjects. The study comparing the disposition kinetics of labeled and unlabeled cotinine (which we will refer to as the cotinine isotope–effects study) was performed in six nonsmokers. Nonsmokers were selected so that there would be no possibility of interference by cotinine derived from nicotine in tobacco. Blood levels of cotinine from environmental tobacco smoke exposure can be found in nonsmokers, but such levels are insignificant compared with those produced by the cotinine infusions in our study. The subjects were three men and three women, ages 27 to 39 (mean age ± SD, 37 ± 5 years), who were determined to be healthy on the basis of screening history, physical examination, and laboratory studies.

The study of the metabolism of nicotine and cotinine was performed in 20 healthy smokers. The subjects were 10 men and 10 women, ages 23 to 51 (mean age, 36 ± 13 years), who had smoked an average of 23 ± 10 cigarettes per day (range, 10 to 50), and who had smoked for an average of 20 ± 14 years (range, 4 to 46 years). U.S. Federal Trade Commission yields for their usual cigarette brands averaged 14.6 ± 4.2 mg tar, 1.0 ± 0.2 mg nicotine, and 14.0 ± 28 mg carbon monoxide. Blood samples for measurement of cotinine concentration were obtained during an outpatient screening visit during which smokers were smoking ad libitum. Samples were generally taken between 10 AM and noon, with a range from 9:10 AM to 3:30 PM.

Experimental protocol. Subjects were studied as outpatients on the General Clinical Research Center at San Francisco General Hospital. All women were studied in the luteal phase of the menstrual cycle. Infusions were performed between 8 and 9 AM after an overnight fast. Smokers were not permitted to smoke during the infusion, but smoking was allowed at other times. Intravenous catheters were placed in the antecubital vein of one arm for infusion and into a forearm vein of the other arm for blood sampling.

Subjects in the cotinine isotope–effects study received a 30-minute infusion of natural cotinine, 4′,4′-dideuterocotinine (cotinine-d_2), and 2,4,5,6-tetradeuterocotinine (cotinine-d_4). The dose of each was 2 μg cotinine base/kg/min. Venous blood samples were collected before, at 15, 30, and 60 minutes after, and at 2, 4, 8, 12, 16, 24, 36, 48, and 72 hours after the beginning of the cotinine infusion.

Subjects in the nicotine-cotinine metabolism study each received a 30-minute infusion of a 50:50 mixture of nicotine-d_2 and cotinine-d_4, each at a rate of 2 μg base/kg/min. Venous blood samples were collected at 0, 10, 20, 30, 45, 60, 90, 120, and 180 minutes and then at 4, 6, 8, 24, 48, 72, and 96 hours after the beginning of the infusion.

Labeled nicotine and cotinine. The syntheses of nicotine-d_2 and cotinine-d_2 have been described previously.[6] Cotinine-d_4 was synthesized by a modification[7] of our previously reported method for the synthesis of cotinine-d_2.[6] Pyridine-d_5 (Schweizerhall, South Plainfield, N.J.; 99.5 atom %D) was converted to 3, 5-dibromopyridine-d_3 by bromination with elemental bromine with use of the method of Garcia et al.[8] With use of the method of Seeman,[9] 3,5-dibromopyridine-d_3 was converted to 5-bromomyosmine-d_3, which was reduced to 5-bromonornicotine-d_3 and resolved to give *(S)*-5-bromonornicotine-d_3, as described previously for the nondeuterated analogs.[10] Reduction of *(S)*-5-bromonornicotine-d_3 to *(S)*-nornicotine-d_4 was accomplished with powdered zinc in deuteroacetic acid/deuterium oxide (1:10 vol/vol) at 0° C. Nornicotine-d_4 was converted to cotinine-d_4 by procedures analogous to our previously reported method for the synthesis of cotinine-d_2.[6] Nicotine and nicotine-d_2 was used as the bitartrate salts.[6] The bitartrate salts were purified by recrystallization from ethanol. Cotinine-d_2 and cotinine-d_4 were purified as their perchlorate salts, crystallized from isopropyl alcohol. The perchlorate salts converted to the free bases with aqueous sodium hydroxide, extracted into methylene chloride and distilled in vacuo. Unlabeled cotinine was used as the fumarate salt.[11] Solutions for injection were made in saline solution, sterilized by autoclaving, and stored in sealed vials under a nitrogen atmosphere. Fig. 1 shows the structures of the labeled compounds used in this study.

Chemical analyses. Concentrations of natural and deuterium-labeled nicotine and cotinine were measured by gas chromatography–mass spectrometry, with use of nicotine-3′,3′-d_2-*N*′-methyl-d_2 (nicotine-

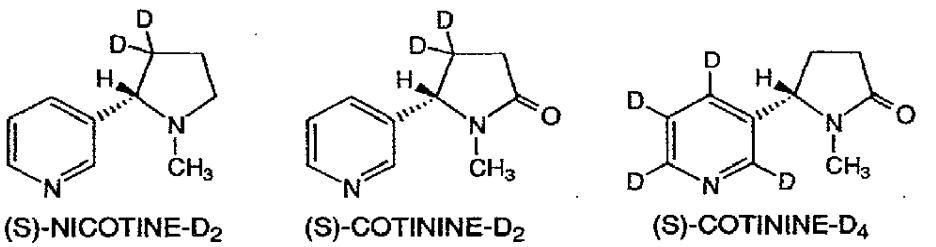

Fig. 1. Structures of nicotine, cotinine, and deuterium-labeled analogs.

d_4) and cotinine-2,4,5,6,4′,4′-d_6-*N*′-methyl-d_3 (cotinine-d_9) as internal standards.[5] The limit of quantitation was 0.5 ng/ml for nicotine isotopomers and 5 ng/ml for cotinine isotopomers. Concentrations of nicotine and cotinine-d_2 were corrected for the presence of naturally occurring stable isotopes in nicotine from tobacco.

Data analysis. Pharmacokinetic parameters for nicotine and cotinine were estimated from plasma concentrations with use of both model-independent methods[12] and a two-compartment open-body model. The area under the blood concentration–time curves (AUC) were computed by the trapezoidal rule. Elimination half-life ($t_{1/2}$) values were computed by linear regression of the log concentration versus time curve in the terminal log-linear phase.

Total clearances (CL) were computed:

$$CL_{nic} = \frac{Dose_{nic\text{-}d_2}}{AUC_{nic\text{-}d_2}}$$

$$CL_{cot} = \frac{Dose_{cot\text{-}d_4}}{AUC_{cot\text{-}d_4}}$$

The fractional conversion of nicotine to cotinine (f) was estimated by use of plasma levels of cotinine generated from infused nicotine-d_2 and the clearance of cotinine-d_4:

$$f = \frac{AUC_{cot\text{-}d_2}}{Dose_{nic\text{-}d_2}} \times CL_{cot}$$

in which AUC and dose are normalized for molecular weights of cotinine and nicotine, respectively.

Daily intake of nicotine from tobacco was computed on the basis of the fractional conversion of nicotine to cotinine and total clearance of cotinine as follows:

$$\text{At steady state, } COT_{gen} = COT_{elim} = f \times D_{nic}$$

in which COT_{gen} and COT_{elim} are the amount of cotinine generated and eliminated per day, f is the fractional conversion of nicotine to cotinine, and D_{nic} is the nicotine dosing rate (i.e., daily intake of nicotine).

The cotinine elimination rate can be estimated as the product of the time-weighted average cotinine concentration ($\bar{C}_{cot}$) and total cotinine clearance (CL_{cot}):

$$COT_{elim} = \bar{C}_{cot} \times CL_{cot}$$

Combining the two previous equations:

$$D_{nic} = \frac{\bar{C}_{cot} \times CL_{cot}}{f} = \bar{C}_{cot} \times K$$

in which K is the conversion factor to convert the time-weighted average plasma cotinine concentration (in nanograms per milliliter) to daily intake of nicotine (in milligrams per 24 hours). Estimation of $\bar{C}_{cot}$ from plasma cotinine levels drawn at various times of day during ad libitum smoking is described below. Using the daily intake of nicotine and the number of cigarettes smoked per day, we also estimated the average nicotine intake per cigarette.

Regression analysis was used to examine relationships among nicotine clearance, cotinine clearance, and f, as well as among cotinine levels, cigarettes per day, and daily intake of nicotine. The student *t* test was used to compare pharmacokinetic data for cotinine-d_2 versus cotinine-d_4 and findings in male and female smokers as well as between smokers and nonsmokers.

Estimation of time-weighted average cotinine concentrations. The subjects in this study had plasma cotinine levels measured at various times of day during ad libitum cigarette smoking. But the computations for estimation of daily intake of nicotine described above require an estimate of the time-weighted average cotinine level. To determine the correction factor to convert a casual plasma level to the time-weighted average level of cotinine, we analyzed data on cotinine levels measured at regular intervals throughout the day during cigarette smoking, collected as part of other studies we have performed. We used the data

Table I. Pharmacokinetics of cotinine and deuterium-labeled analogs

Subject No.	Body Weight (kg)	Plasma clearance (ml/min)			V_{SS} (L)			$t_{1/2}$ (min)		
		Cotinine	Cot-d_2	Cot-d_4	Cotinine	Cot-d_2	Cot-d_4	Cotinine	Cot-d_2	Cot-d_4
1	86.9	37.7	37.7	36.5	56.5	55.6	53.5	1081	1061	1056
2	64.0	32.9	32.4	30.4	66.4	64.2	64.0	1446	1416	1490
3	58.0	47.5	45.6	44.4	46.4	47.6	45.5	731	788	778
4	76.5	63.0	56.6	58.1	68.3	71.9	68.5	840	961	909
5	94.0	74.7	62.7	69.5	74.0	68.1	72.6	770	814	812
6	60.0	33.4	30.0	31.7	45.8	47.6	45.3	985	1138	1029
Mean		48.2	44.2	45.1	59.6	59.2	58.3	976	1030	1012
SD		17.2	13.3	15.7	11.9	10.5	11.8	266	233	259

V_{SS}, Steady-state volume of distribution; $t_{1/2}$, half-life; Cot-d_2, cotinine-d_2; Cot-d_4, cotinine-d_4.

from 31 smokers who participated in three different studies. The subjects smoked an average of 22 cigarettes per day (range, 8 to 37). Plasma cotinine levels were sampled every 4 hours for 24 hours. The time-weighted average cotinine concentrations were computed as the area under the plasma cotinine concentration-time curve over 24 hours, divided by 24 hours. For each subject, the ratio of the actual plasma cotinine concentration at various times of day and a time-weighted average cotinine was computed. The ratios were then averaged for all subjects. These ratios with appropriately interpolated values were then used to correct the measured plasma cotinine (C_{cot}) concentration as follows:

$$\text{Estimated } \bar{C}_{cot} = C_{cot}/\text{ratio}$$

RESULTS

Cotinine isotope–effect study. Mean plasma concentrations for natural cotinine, cotinine-d_2, and cotinine-d_4 during and after infusion were virtually superimposable. The average plasma concentration for the three compounds at the end of the infusion were 118, 115, and 116 ng/ml, respectively. Pharmacokinetic parameters were similar for natural and labeled cotinine analogs (Table I).

Disposition kinetics of nicotine and cotinine in smokers. Plasma levels of nicotine-d_2, cotinine-d_2, and cotinine-d_4 in smokers after infusion of a 50:50 mixture of nicotine-d_2 and cotinine-d_4 are shown in Fig. 2. Plasma nicotine-d_2 and cotinine-d_4 at the end of the infusion averaged 24.5 ng/ml (SD, 9.0) and 86.4 ng/ml (SD, 27.0), respectively. Cotinine-d_2, derived from nicotine-d_2, peaked at an average concentration of 40.3 ng/ml (SD, 7.4), at 240 minutes after initiation of the infusion. Pharmacokinetic parameters for individual subjects are shown in Fig. 3, with average values provided in Tables II and III. The $t_{1/2}$ values of cotinine-d_2 (derived from nicotine-d_2) and of cotinine-d_4 were similar, on average, and were highly correlated ($r = 0.91$).

On average, 72% of nicotine was converted to cotinine, with a range from 55% to 92% (Table III). Subjects with low clearances of nicotine tended to have low fractional conversions of nicotine to cotinine. For example, the subject with the lowest clearance of nicotine (526 ml/min) had one of the lowest fractional conversions of nicotine to cotinine (59%). Considering all subjects, the fractional conversion of nicotine to cotinine was significantly correlated with nicotine clearance ($r = 0.59$; $p < 0.05$; Fig. 4). The clearances of cotinine-d_4 and nicotine-d_2 were also significantly correlated ($r = 0.91$; $p < 0.001$). No significant gender-related differences in pharmacokinetic parameters were observed. Pharmacokinetic parameters for cotinine-d_4 were similar for nonsmokers who received cotinine-d_4 in the cotinine isotope effect study and smokers who received cotinine-d_4 in the nicotine metabolism study.

Parameters for estimation of time-weighted average cotinine concentration. Fig. 5 shows average plasma cotinine concentration throughout the day in 31 cigarette smokers. The relationship between plasma cotinine concentration at different times of day and the time-weighted average are shown in Table IV Based on these ratios, the screening plasma cotinine levels were converted to the predicted time-weighted average plasma cotinine concentration, as shown in Table V. These corrected values were then used for computation of daily intake of nicotine as described previously.

Nicotine intake during cigarette smoking. The plasma cotinine concentration measured during ad libitum smoking at the screening visit for the study was used for computation of daily intake of nicotine. As

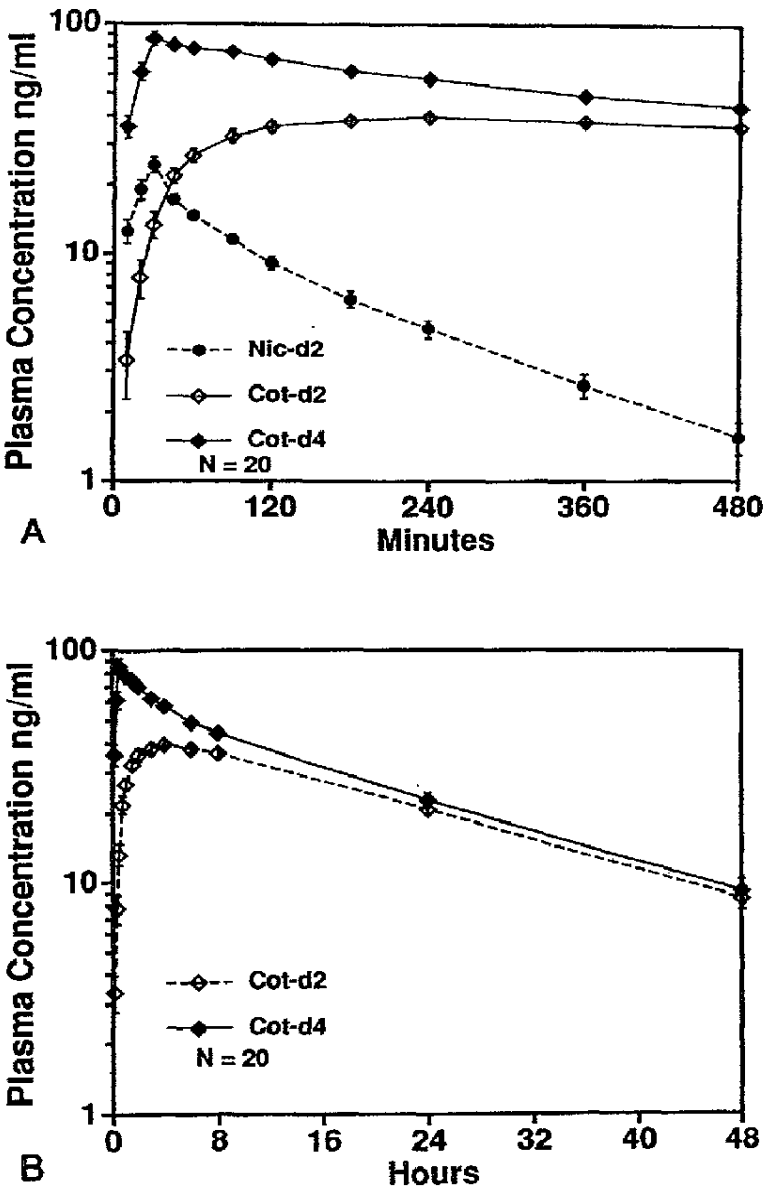

Fig. 2. Mean plasma concentrations of nicotine-d_2, cotinine-d_2, and cotinine-d_4 during and after intravenous infusion of a 50:50 mixture of nicotine-d_2 and cotinine-d_4 (2 μg base/kg/min of each for 30 minutes, beginning at time zero). **A,** Shows the values up to 480 minutes. **B,** Shows values up to 48 hours. Data are presented only through 48 hours because beyond that time cotinine concentrations for many subjects were below the limit of quantitation. *Curves* represent average values for 20 subjects.

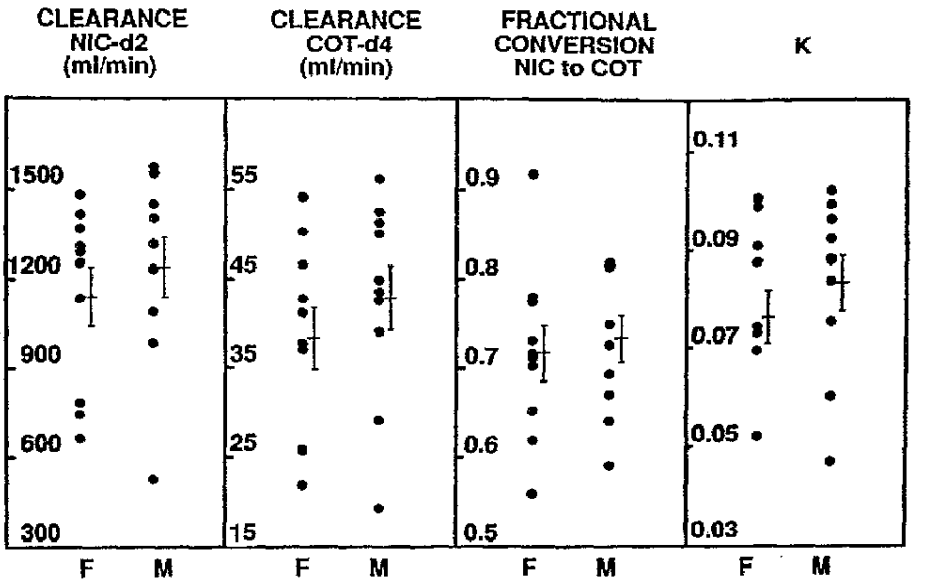

Fig. 3. Nicotine and cotinine plasma clearance, fractional conversion of nicotine to cotinine, and the factor that converts plasma cotinine concentration to daily intake of nicotine (K). Data are shown for individual subjects by gender. *Bars* indicate mean ± 95% confidence intervals.

Table II. Pharmacokinetics of nicotine-d_2

	Body weight (kg)	*Plasma clearance*		V_{SS}		
		ml/min	*ml/min/kg*	*L*	*L/kg*	$t_{1/2}$ *(min)*
Men (n = 10)						
Mean	75.8	1237	16.7	200	2.7	142
Range	61.3-100.4	526-1573	8.3-25.7	156-273	2.0-4.2	104-227
CV (%)	16.9	25.4	32.1	17.0	25.0	25.2
Women (n = 10)						
Mean	65.3	1145	17.7	186	2.9	135
Range	53.0-94.3	662-1484	11.3-25.8	126-242	2.1-4.0	95-199
CV (%)	18.7	26.5	26.2	19.9	22.2	27.2
All subjects (n = 20)						
Mean	70.5	1191	17.2	193	2.8	138
Range	53.0-100.4	526-1573	8.3-25.8	126-273	2.0-4.2	95-227
CV (%)	18.9	25.5	28.5	18.4	23.2	25.6

V_{SS}, Steady-state volume of distribution; $t_{1/2}$, elimination half-life; CV, Coefficient of variation = SD/mean.

seen in Table IV, the average daily nicotine intake was 20.2 mg (range, 4.4 to 37.1 mg), with an average nicotine intake per cigarette of 0.87 mg (range, 0.22 to 1.92 mg). Men had a significantly greater intake of nicotine per day, because they smoked more cigarettes per day. There was no gender-related difference in nicotine intake per cigarette. The factor that converts cotinine concentration to daily intake of nicotine averaged 0.08 (range, 0.047 to 0.102).

Correlations were significant between daily intake of nicotine during cigarette smoking versus cigarettes per day ($r = 0.57$; $p < 0.01$) and versus plasma cotinine concentration ($r = 0.82$; $p < 0.001$). Plasma cotinine concentration and cigarettes per day were also significantly correlated ($r = 0.44$; $p = 0.05$). Machine-determined nicotine yields and nicotine intake per cigarette were not significantly correlated ($r = 0.10$).

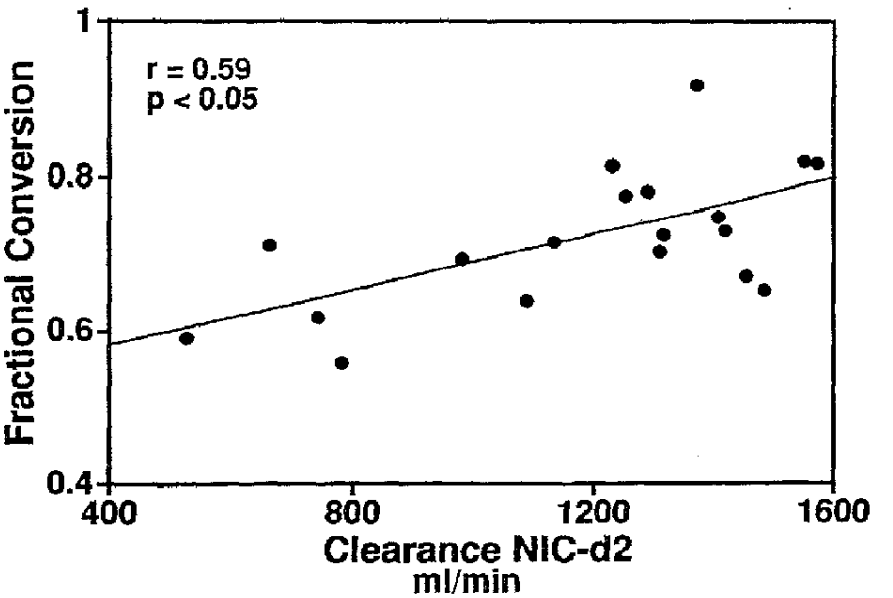

Fig. 4. Scatter plot and regression line between plasma clearance of nicotine-d_2 and the fractional conversion of nicotine to cotinine (f). The regression equation is $f = 0.51 + 0.18\ CL_{nic\text{-}d_2}$ (L/min).

Table III. Pharmacokinetics of cotinine and conversion of nicotine to cotinine

	Cotinine-d_4 plasma clearance		*Cotinine-d_4 V_{SS}*				
	ml/min	*ml/min/kg*	*L*	*L/kg*	*Cot-d_4 $t_{1/2}$ (min)*	*Cot-d_2 $t_{1/2}$ (min)*	*f*
Men (n = 10)							
Mean	42.9	0.58	61.8	0.84	1141	1278	0.73
Range	19.3-56.2	0.30-0.87	47.3-86.6	0.60-1.35	782-1760	882-2334	0.59-0.82
CV (%)	26.5	34.0	18.9	26.5	24.5	32.5	11.4
Women (n = 10)							
Mean	38.3	0.60	45.9	0.69	953	1084	0.72
Range	21.9-54.2	0.36-0.90	33.4-87.0	0.55-0.92	488-1563	543-1643	0.56-0.92
CV (%)	28.6	32.0	34.8	17.7	32.5	30.8	13.7
All Subjects (n = 20)							
Mean	40.6	0.59	54.0	0.76	1047	1181	0.72
Range	19.3-56.2	0.30-0.90	33.4-87.0	0.55-1.35	488-1760	543-2334	0.55-0.92
CV (%)	27.4	32.2	29.5	24.7	29.0	32.2	12.3

V_{SS}, Steady-state volume of distribution; $t_{1/2}$, elimination half-life; f, fractional conversion of nicotine to cotinine.

DISCUSSION

Using stable isotope methods, we have characterized the disposition kinetics of nicotine and cotinine, including the fractional conversion of nicotine to cotinine, in 20 smokers. This study is the first to explictly determine the extent of nicotine metabolism to cotinine. A novel method for estimating daily intake of nicotine from cigarette smoking is described and a quantitative perspective on the accuracy and sources of error in the use of plasma cotinine to estimate nicotine intake from smoking is provided.

Before labeled analogs to probe drug disposition are used, it is necessary to validate the assumption that the disposition kinetics of the natural and labeled compounds are the same. In a previous study, we have shown that this is so for nicotine and nicotine-d_2.[5] In the present study, we confirmed the lack of an isotope effect for cotinine-d_2 and cotinine-d_4 (i.e., similar

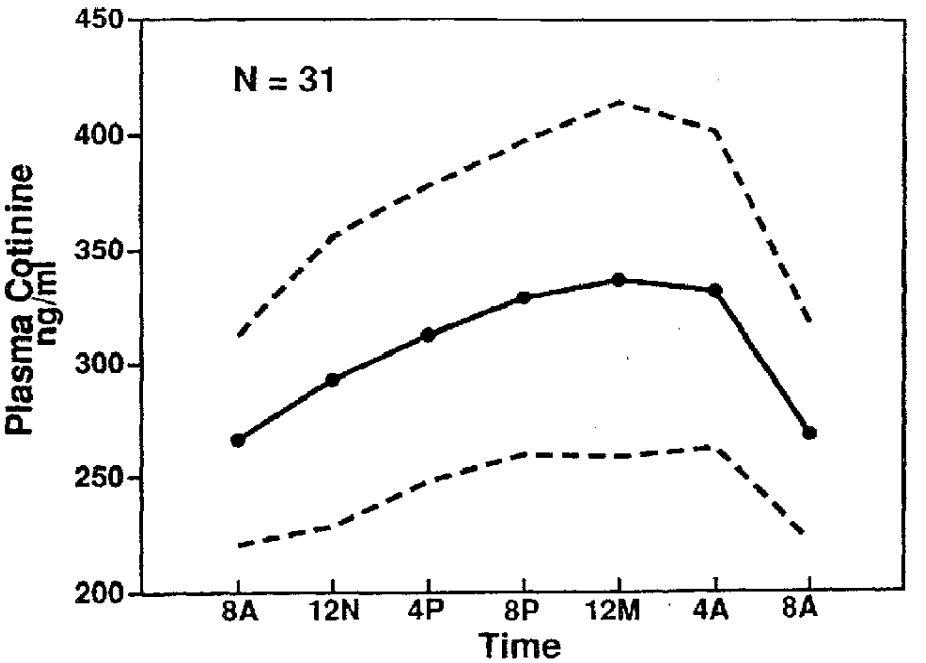

Fig. 5. Average plasma cotinine concentrations at various times of day in 31 smokers who smoked an average of 22 cigarettes per day (range, 8 to 37). *Broken lines* represent 95% confidence intervals.

Table IV. Time of sampling of plasma cotinine concentration and its relationship to the time-weighted average plasma cotinine concentrations in cigarette smokers (n = 31)

Time of day (hr)	*Measured $C_{cot}/\bar{C}_{cot}$ ratio*	*95% CI*
8 AM	0.90	0.84-0.96
9 AM*	0.90	—
10 AM*	0.91	—
11 AM*	0.91	—
Noon	0.92	0.88-0.96
1 PM*	0.94	—
2 PM*	0.96	—
3 PM*	0.97	—
4 PM	0.99	0.96-1.02
5 PM*	1.00	—
6 PM*	1.02	—
7 PM*	1.03	—
8 PM	1.05	1.02-1.08
9 PM*	1.06	—
10 PM*	1.06	—
11 PM*	1.07	—
Midnight	1.08	1.02-1.13
4 AM	1.06	1.02-1.10

C_{cot}, Measured plasma cotinine concentration; $\bar{C}_{cot}$, time-weighted average plasma cotinine concentration; CI, confidence interval.
To estimate $\bar{C}_{cot}$ for any individual smoker: $\bar{C}_{cot} = C_{cot}$/ratio.
*Interpolation.

elimination kinetics for natural and labeled cotinine). These studies were performed in nonsmokers so that there would be no interference by natural cotinine derived from nicotine in tobacco. Because we also studied a group of smokers, we are able to compare the kinetics of cotinine-d_4 in smokers and nonsmokers. The disposition kinetics of cotinine were similar in these two groups, consistent with our observations made previously based on the $t_{1/2}$ of cotinine alone[13] and as suggested by other investigators who infused cotinine into nonsmokers and reported pharmacokinetic parameters similar to those reported by other investigators in smokers.[14,15]

The average pharmacokinetics for nicotine in this study were similar to those reported in a previous study with infusion of nicotine-d_2[4] and similar to other studies using unlabeled nicotine.[16,17] Our data on cotinine kinetics differ somewhat from our earlier study with the infusion of natural cotinine in smokers.[11] In the present study, the average clearance and volume of distribution are about one-third lower. The $t_{1/2}$ values in both studies are similar, averaging 16 to 17 hours. Other recent studies on cotinine kinetics report clearance data similar to that of our present study.[14,15] We cannot explain why our early data and our more

Table V. Nicotine intake during cigarette smoking

	C_{cot} (ng/ml)	$\bar{C}_{cot}$ (ng/ml)	Cigarettes per day	K ($\times 10^{-3}$)*	D_{nic} (mg/24 hr)†	Nicotine intake per cigarette (mg)†
Mean (n = 10)						
Mean	342	317	27.5	83.2	25.5‡	0.91
Range	103-705	98-642	15-40	47.1-101.9	8.2-37.1	0.36-1.48
CV (%)	51.8	51.4	23.1	21.2	44.9	40.6
Women (n = 10)						
Mean	218	200	20.2	76.3	14.8‡	0.84
Range	66-374	60-340	10-50	52.2-100.6	4.4-24.2	0.22-1.92
CV (%)	46.7	46.0	55.3	22.9	44.6	58.2
All subjects (n = 20)						
Mean	280	258	23.8	79.8	20.2	0.87
Range	66-705	60-642	10-50	47.1-101.9	4.4-37.1	0.22-1.92
CV (%)	55.2	54.9	40.2	21.9	52.7	48.3

C_{cot}, Screening plasma cotinine; $\bar{C}_{cot}$, estimated time-weighted average plasma cotinine; K, conversion factor; D_{nic}, daily nicotine intake.
*Conversion factor based on equation: D_{nic} (mg/24 hr) = Plasma Cot (ng/ml) × K; (K = CL_{cot}/f (ml/min) × 1.44 × 10^{-3}. See Methods section for derivation).
†Based on estimated time-weighted average plasma cotinine concentration.
‡Men versus women, difference, $p < 0.05$.

recent data differ somewhat, but it is most likely that our recent data, based on a considerably larger number of subjects, are most representative of the population.

We found that an average of 72% of nicotine is converted to cotinine, although there is considerable individual variability—from 55% to 92%. The average of 72% is close to that estimated previously by us based on measurements of renal clearance and urinary recovery of cotinine and nicotine-1′-*N*-oxide[18] and on a recent study based on absolute urinary recovery of cotinine and its metabolites.[19] The known proximate metabolites of nicotine are cotinine, nicotine-1′-*N*-oxide, and normicotine. The latter two account for only a small percentage of nicotine metabolism (3.7% ± 0.9% for nicotine-1′-*N*-oxide and 0.65% ± 0.15% for normicotine).[19] The identity of the proximate metabolites for those subjects who convert relatively low proportions of nicotine to cotinine (two subjects converted <60%) remains to be determined.

It was noted that subjects who converted lesser amounts of nicotine to cotinine appeared to have lower clearance of nicotine. A significant positive correlation between fractional conversion of nicotine to cotinine and clearance of nicotine was confirmed (Fig. 4). Thus the enzymatic conversion of nicotine to cotinine appears to be the most rapid of the various proximate metabolic pathways for nicotine metabolism.

Also of interest is the finding that the clearance of nicotine and cotinine are significantly correlated (Fig. 6). This correlation is somewhat surprising because nicotine is a rapidly metabolized drug, the clearance of which appears to a considerable degree to be hepatic blood flow limited,[20] whereas cotinine is a slowly metabolized drug, the metabolism of which is expected to be limited by enzymatic activity of the liver. In any case, the possibility that the same enzyme is involved in the metabolism of nicotine and cotinine is suggested by these data.

We present a novel method for estimating daily intake of nicotine based on plasma cotinine levels during ad libitum smoking, data on cotinine clearance, and fractional conversion of nicotine to cotinine. A major assumption in this computation is that plasma cotinine levels represent steady-state levels. Plasma cotinine does vary throughout the day with regular smoking, with average fluctuations of 30% from peak to trough. With regular smoking throughout the day, evening plasma cotinine levels are most representative of average levels. Sampling at other times of day may lead to underestimation or overestimation of average cotinine level. For this reason, we analyzed some of our previously collected data to develop correction factors, allowing cotinine values obtained at various times of day to be converted to estimated time-weighted averages or steady-state concentrations. Such a correction with average values implies that all smokers smoke with the same pattern throughout the day and that the disposition kinetics of cotinine are the same. Obviously, this is not the case; however, we believe that making a temporal correction does improve the estimation of nicotine intake

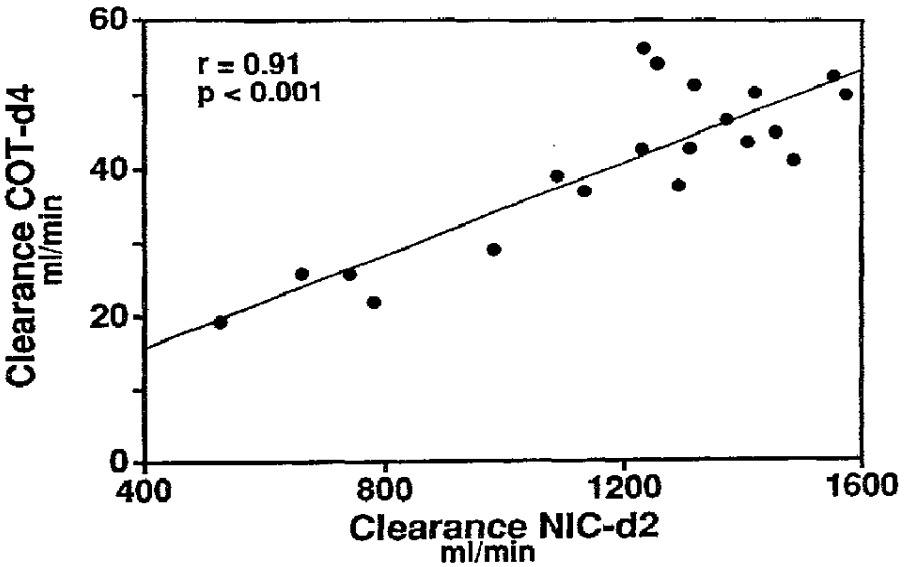

Fig. 6. Scatterplot and regression line between plasma clearance of cotinine-d_4 and nicotine-d_2. The regression equation is $CL_{cot\text{-}d_4}$ (L/min) = 0.003 + 0.031 $CL_{nic\text{-}d_2}$ (L/min).

compared with using cotinine levels obtained at different times of day without correction. Also, day-to-day variability in nicotine intake, such that steady state is not achieved, is another source of error. We also assume that the clearance of nicotine and cotinine are stable in an individual from day to day. We have unpublished evidence that this is the case for nicotine.

With these limitations, our method does provide estimates of nicotine intake and intake per cigarette that are similar to those previously estimated based on circadian measurement of plasma nicotine concentration during cigarette smoking and nicotine clearance.[17,21,22] Similar to previous studies, we found an average intake of nicotine per cigarette of about 1 mg, and virtually no correlation between machine-determined nicotine yield and actual yield of nicotine per cigarette.

Plasma cotinine (as well as salivary or urinary cotinine) have been widely used as biochemical markers of nicotine intake from tobacco.[2,3,23] Our data provide a basis to quantitatively examine the accuracy of cotinine measurement for this purpose. As shown in the equations in the method section, the factor that converts plasma cotinine to daily intake of nicotine is determined by the clearance of cotinine and the fractional conversion of nicotine to cotinine for that individual. The average conversion factor (K) for all of our subjects was 0.08, meaning that every 100 ng/ml cotinine at steady state represents a daily intake of 8 mg nicotine. Thus an average cotinine concentration of 300 ng/ml, reported in a typical population of smokers, corresponds to average daily intake of nicotine of 24 mg.

Unfortunately, there is more than a twofold variability in this conversion factor, ranging from 0.047 to 0.102. Thus a given plasma level of cotinine could result from a twofold different level of nicotine intake in different individuals. Examination of the source of variability in K indicates that more individual variability derives from differences in clearance of cotinine (coefficient of variation, 27.5%) than in fractional conversion of nicotine to cotinine (coefficient of variation, 12.3%). The extent of individual variability in the plasma cotinine–nicotine intake conversion factor explains why the performance of blood cotinine level per se as a predictor of daily intake of nicotine has been limited ($r = 0.53$).[21]

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